# THE ACTION OF B. TYPHOSUS ON XYLOSE AND SOME OF THE OTHER LESS FREQUENTLY USED SUGARS

WITH ONE PLATE

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The bacillus of typhoid fever was discovered early in the course of the development of the science of bacteriology and has been subjected to intensive study by a great number of workers. It has come to be generally regarded as being possessed of greater stability and uniformity with regard to most of its properties than many other pathogenic bacteria, some of which have been subdivided into varieties by means of immunity reactions, fermentation tests, or morphologic differences; the typhoid bacillus is still described in our textbooks as being homogeneous or of a single type.

In 1917, Weiss[1] found that 6 of 31 typhoid strains investigated by him did not produce acid in xylose broth; 4 of these appeared to be also atypical antigenically in that they did not absorb out agglutinins for the xylose-fermenting typhoid strains. The "Rawlings" strain, which has been used so extensively in the preparation of typhoid vaccine, was one of those that did not ferment xylose. This fact appeared to render the question as to whether there are two distinct groups of typhoid bacilli one of practical significance, for, if such is the case, our army was being vaccinated with a strain of the minority type. Shortly after the publication of Weiss' work, Teague and McWilliams[2] showed that by plating and selecting different colonies of a single culture of B. typhosus, similar differences with regard to the absorption of agglutinins could be observed at times with these subcultures as were obtained by Weiss when using his xylose-fermenting and nonfermenting strains. It was suggested that differences in physical aggregation in cultures with varying tendencies toward spontaneous agglutination might, perhaps, cause differences in the absorption of agglutinins in the complete absence of specific antigenic differences in the strains tested. The fact that only four of the six

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[1] Jour. Med. Research, 1917, 31, p. 135.
[2] Jour. Immunol. 1917, 2, p. 383.

nonfermenting strains of Weiss were observed to behave atypically with regard to the absorption of agglutinins made it appear doubtful that these strains really differed immunologically from other typhoid strains; the evidence offered did not lead to the addition of other strains of B. typhosus to the vaccine prepared at the Army Medical School.

In 1918, strains of typhoid that did not ferment xylose were encountered in blood and stool cultures of certain typhoid patients among the American soldiers in France and the interesting observation was made that patients infected with the nonfermenting type of B. typhosus sometimes occurred in definite small groups. An investigation of these cultures, particularly with regard to their immunologic relationship to the xylose fermenting typhoids, is being carried out at the Army Medical School by the officers who collected the cultures in France. The question as to whether additional strains of B. typhosus should be used along with the "Rawlings" strain in the preparation of vaccine thus again arose, and Col. F. F. Russell directed us to investigate a larger number of cultures than had been used by Weiss with regard to their ability to ferment xylose. It was thought advisable to study at the same time the action of B. typhosus on some of the other less frequently used sugars. We decided to study the action of the growing bacilli both in fluid medium and when inoculated on the surface of agar plates containing the sugar and a suitable indicator for acid-production.

We shall first present the results of our experiments and will then try to point out in what respects they are confirmatory of results previously obtained by other workers and in what respects they supplement those previous observations.

The cultures of B. typhosus used in this investigation may be considered in three groups:

1. The collection of the Army Medical School, consisting of 116 strains. The sources of these cultures were as follows:

| | |
|---|---|
| Blood cultures | 40 strains |
| Stool cultures | 11 strains |
| Urine cultures | 11 strains |
| Bile cultures | 1 strain |
| Source not recorded | 53 strains |

The cultures had been carried on artificial mediums for the following lengths of time:

| | |
|---|---|
| More than two years | 5 strains |
| Between one and two years | 25 strains |
| Between six months and one year | 36 strains |
| Less than six months | 32 strains |
| Isolated during this investigation | 11 strains |
| Age not recorded | 7 strains |

Of the 116 strains, 94 were obtained from army camps in the United States, 9 from camps in France, and the majority of the remaining 12 from civilian hospitals in the United States.

Two of the strains, namely, Pierce and Lelito, were recovered from chronic typhoid carriers. Numbers 25 and 33 are duplicate cultures obtained from Lelito at different times and 34 is a duplicate culture obtained from Pierce.

One of the strains is the well-known "Rawlings" strain, which has been used for many years in England and the United States for the preparation of typhoid vaccine. It was obtained for this collection from the Royal Army Medical School, England, in March, 1908.

2. A group of older cultures, consisting of 10 strains. Three of these had been obtained from Dr. J. C. Torrey of the Cornell University Medical School, New York, more than 5 years ago; 5 were isolated by one of us (Teague) about 3 years ago at the Quarantine Laboratory, Port of New York; and 2 came from Boston. Four of the 5 cultures from the Quarantine Laboratory were from blood cultures, the other from a stool culture; the 2 cultures from Boston were obtained from blood cultures.

3. A group of 12 cultures obtained from R. C. Colwell, 1st Lieut. Sanitary Corps, U. S. Army, through the courtesy of Lieut.-Col. H. J. Nichols. It was stated that these cultures did not ferment xylose and that they had been selected by Lieut. Colwell, precisely on account of this property, from a large number of cultures of B. typhosus obtained from American army camps in France. The 12 strains were derived from 9 patients, cultures from both blood and stools of 3 patients having been included. The duplicate strains were the following:

| | |
|---|---|
| C-175 | Blood culture |
| C-176 | Stool culture |
| C-48 | Blood culture |
| C-59 | Stool culture |
| C-188 | Blood culture |
| C-189 | Stool culture |

Six of these 12 cultures were isolated between Nov. 22, 1918, and Feb. 6, 1919.

## RESULTS OF EXPERIMENTS

*Xylose.*—We shall consider first the results of the inoculation of the cultures of groups 1 and 2 into xylose broth. These cultures came from widely separated districts and varied greatly as to the length of time they had been carried on artificial culture mediums. The proportion of strains that do not ferment xylose found among them should represent, therefore, approximately the proportion in which such strains actually occur.

April 19, 1919, in the first tests; 115 cultures were included. The medium consisted of 1% peptone, 1% nutrose, 1% xylose and 0.5% sodium chlorid with litmus as an indicator. Two c c amounts were placed in small test tubes and sterilized in the autoclave at 10 lbs. pressure for 10 minutes. Peptone-water tubes were inoculated with the cultures and, after they had been incubated over night, one loopful of the peptone water growth was transferred to each xylose broth tube; the latter were then kept in the incubator until the close of the experiment. Endo plates were inoculated from the xylose broth cultures in the hope of detecting any accidental contaminations that may have taken place during the inoculation of the tubes. The result of these first tests was as follows—"positive" indicated that the medium became unmistakably acid, "negative" that the medium remained neutral or became alkaline:

| | |
|---|---|
| Positive in 24 hours | 106 cultures |
| Positive on the 5th day (57 and 156) | 2 cultures |
| Positive on the 6th day (Rawlings and Wright) | 2 cultures |
| Positive on the 7th day (K-9) | 1 culture |
| Positive on the 8th day (77) | 1 culture |
| Negative on the 27th day (49, 75 and Jones) | 3 cultures |

Thus, 9 of the 115 cultures did not ferment the xylose or fermented it very slowly; however, when transplants of these 9 cultures were made from the xylose broth tubes on the eleventh day to new xylose-broth tubes, all of them with the exception of Wright showed acid production in 24 hours.

April 25, 1919, in the second tests, the same cultures as in the preceding tests were employed, and the xylose broth was prepared in the same way. The results were as follows:

| | |
|---|---|
| Positive in 24 hours | 107 cultures |
| Positive on the 5th day (57) | 1 culture |
| Positive on the 8th day (49, 77, Rawling) | 3 cultures |
| Positive on the 10th day (Wright) | 1 culture |
| Positive on the 11th day (75 and Jones) | 2 cultures |
| Positive on the 21st day (K-9) | 1 culture |

Culture 156, a slow fermenter of the previous tests, was found to be contaminated in this series and is not included; with this exception, the negative cultures and slow fermenters of the first series correspond exactly to the slow fermenters of the second series of tests. Two other cultures, 10 and 163-D, showed only questionable acidity in 24 hours, but were strongly acid, the one in 48 hours and the other on the third day.

May 17, 1919, in the third tests, the same cultures were again used, but the medium was prepared from meat infusion rendered sugar-free by inoculation with B. coli, to which was added 1% peptone, 1% xylose and 0.5% sodium chlorid. Litmus was the indicator. The results were as follows:

| | |
|---|---|
| Positive in 24 hours | 107 cultures |
| Positive on the 7th day (57) | 1 culture |
| Negative on the 16th day (49, 75, 77, 156, Jones, Wright, Rawlings and K-9) | 8 cultures |

The negative and slowly fermenting cultures correspond to the negative and slowly fermenting cultures of the two preceding series of tests.

June 26, 1919, in the fourth tests, freshly isolated cultures of B. typhosus, which were not included in the preceding tests, were employed. The medium contained 0.25% nutrose, 1% peptone, 0.5% sodium chlorid and 1% xylose. Phenol red and decolorized china blue (Morishima[3]) were used as indicators. The results were as follows:

| | |
|---|---|
| Positive in 24 hours | 9 cultures |
| Negative on the 32nd day (Brockney) | 1 culture |

A total of 126 cultures of B. typhosus were examined with regard to their ability to ferment xylose; 92% of them fermented the xylose promptly, the other 8% produced an acid reaction in the xylose-broth not at all, or only after a number of days' incubation.

The histories of the negative cultures, so far as they are available, are in table 1.

TABLE 1

HISTORIES OF CULTURES THAT DO NOT FERMENT XYLOSE OR FERMENT IT VERY SLOWLY

| Number of Culture | Date Received at the Army Medical School | Source | Material Cultured | Approximate Age of Culture |
|---|---|---|---|---|
| 49 | Aug. 23, 1918 | Columbus Barracks, O. | Blood | 8 months |
| 57 | Aug. 3, 1918 | Camp Logan, Texas | Blood | 9 months |
| 75 | Aug. 29, 1918 | Camp Travis, Texas | ..... | 8 months |
| 77 | Aug. 30, 1918 | Camp Dodge, Ia. | Blood | 8 months |
| 156 | Feb. 20, 1919 | Camp Eustis, Va. | Blood | 2 months |
| Jones | 1915 | ..... | ..... | 4 years |
| Wright | 1909 | From Sir A. E. Wright, London, England | ..... | 10 years |
| Rawlings | 1908 | From the Royal Army Medical College, England | ..... | 11 years |
| Brockney | June 6, 1919 | Port of Embarkation, Hoboken, N. J. | Blood | a few days |
| K-9 | ..... | From Dr. J. C. Torrey, Cornell University Medical School, New York | ..... | 6 years |

[3] Morishima, this journal, preceding article.

It is seen that the failure to ferment xylose promptly is not due to long continued cultivation of the B. typhosus on artificial mediums. No evidence is afforded in favor of the view that infections with the atypical typhoid bacillus are particularly frequent in certain districts.

June 26, 1919, in the fifth series of tests cultures of group 3 were used, which were selected on account of not fermenting xylose from a large number of cultures obtained in France. The medium contained 0.25% nutrose, 1% peptone, 0.5% sodium chlorid and 1% xylose. Phenol red and decolorized china blue served as the indicator.

| | |
|---|---|
| Positive in 24 hours | 0 cultures |
| Positive on the 6th day (C-51) | 1 culture |
| Positive on the 8th day (C-49 and C-175) | 2 cultures |
| Positive on the 14th day (C-60, C-176 and C-195) | 3 cultures |
| Positive on the 18th day (C-50 and C-189) | 2 cultures |
| Negative on the 32nd day (C-48, C-59, C-183 and C-188) | 4 cultures |

It is seen that the statement that these cultures do not ferment xylose is not, strictly speaking, correct. Only 4 of the cultures failed to call forth an acid reaction in the xylose broth and one of them produced acid as early as the sixth day.

July 4, 1919, in the sixth series of tests, the same cultures were used as in the preceding tests. The medium consisted of meat infusion rendered free from sugar by inoculation with B. coli, to which were added 1% peptone, 0.5% sodium chlorid and 1% xylose. Phenol red and decolorized china blue served as the indicator.

| | |
|---|---|
| Positive in 24 hours | 0 cultures |
| Negative on the 25th day | 12 cultures |

*Xylose-agar plates.*—In the preceding experiments the phenol red and decolorized china blue in combination served as a very satisfactory indicator in the xylose-broth. The phenol red was added to the broth and the reaction was adjusted to $P_H = 7.1$; then the decolorized china blue was added. The medium was usually almost colorless, but if the reaction had been improperly adjusted it had a greenish or pinkish tinge; even then it was quite satisfactory if a control uninoculated tube was used for comparison. A small amount of acid turned the medium bright green, a larger amount made it deep blue; alkali made the medium pink. The slowly fermenting typhoid bacilli almost invariably called forth an alkaline reaction in the medium during the first few days of incubation, and consequently the contrast between the pink color of the negative cultures and the green or blue color of the positive ones was most striking. It was decided to use the decolorized china blue as an indicator in the xylose agar also, the nutrient agar having been first adjusted to approximately $P_H = 7.1$ by the use of phenol red. We also employed the methylene blue-eosin medium of Holt-Harris and Teague.[4] Both of these plates remain unaffected by light and hence offered a distinct advantage over the Endo plate in these experiments, where the period

[4] Jour. Infect. Dis., 1916, 18, pp. 596-600.

of observation was often one or two weeks and sometimes three weeks. The nutrient agar employed contained 1% peptone, 0.33% Liebig's beef extract and 0.5% sodium chlorid; xylose to the amount of 1% and an appropriate amount of the indicators were added to the sterile melted agar, which was heated in the autoclave for 10 minutes at 10 lbs. pressure and then poured into petri dishes.

When typhoid cultures that ferment xylose rapidly are inoculated on the xylose-china-blue plate, large blue colonies develop in 24 hours; these colonies increase in size and intensity of color during the succeeding days but show no changes of interest when observed for 10 days or 2 weeks. Usually the isolated colonies are all of the same color, but occasionally some are paler during the first day or two of incubation. The color diffuses into the medium around the colonies, and the whole plate soon becomes blue. When the rapidly fermenting cultures are inoculataed on methylene blue-eosin xylose plates, the isolated colonies show black centers by transmitted light after 24 hours' incubation; on the succeeding days the black centers become larger, but the colonies offer no developments of interest over long periods of observation. Occasionally some of the isolated colonies did not have black centers after 24 hours' incubation, but when such colonies were fished and inoculated into xylose broth, they invariably caused good acid production in 24 hours; this was also true of the paler colonies on the china plates. It seemed possible that some of the cultures, which fermented xylose in broth rapidly, might contain slowly fermenting typhoid bacilli together with the rapidly fermenting ones. Consequently 38 different strains of B. typhosus which had been found to ferment xylose rapidly in broth were inoculated on the china blue-xylose plate, on the methylene blue-eosin-xylose plate, or on both. Not a single colony that was fished from the plate, yielded a slowly fermenting culture when transplanted to xylose broth. Hence the conclusion seems warranted that the rapidly fermenting strains of B. typhosus are homogeneous; i. e., they consist solely of rapidly fermenting individuals even though the culture may have been carried on agar slants for long periods of time.

If typhoid cultures that ferment xylose slowly are inoculated on xylose-china-blue plates, colorless colonies develop, which after 24 hours' incubation are somewhat thinner and consequently more transparent than colonies on control plates without xylose. On the second day, or somewhat later according to the culture used, many very small daughter colonies appear within the isolated colonies on the plate. The

daughter colonies rapidly become much more opaque than the colony in which they are developing and project above the surface of the main colony as papillae. Each isolated colony on the plate may contain a dozen or more of the daughter colonies. If such a plate is viewed by transmitted light on the fifth or sixth day, the rather delicate typhoid colonies speckled with round opaque daughter colonies present an appearance entirely foreign to that of the usual typhoid colonies. A few days later deep blue areas appear in a few of the colonies; these are obviously daughter colonies that are producing acid from the xylose in the culture medium. The blue daughter colonies frequently grow with surprising rapidity and become much larger than the colony in which they originated. They yield a heaped up growth, which is quite different in appearance from the usual flat typhoid colony. In the succeeding days other colonies may show blue daughter colonies, which in turn soon develop into the large heaped up growths; later the china blue in some of the large colonies may become reduced giving the growth a brownish yellow color. Any one unfamiliar with the phenomenon under consideration would regard such a typhoid plate with large daughter colonies as being surely contaminated with other bacteria. The number of large blue colonies that develop on the plate varies greatly according to the strain of B. typhosus employed; sometimes only 1, 2 or 3 appear, in other instances 50, or 100 or more may develop. It is our custom to inoculate one edge of the plate with a loopful of a 24 hour peptone water culture of B. typhosus and then gradually spread the material over the rest of the plate; the developing colonies give a confluent growth over about one-third of the plate and yield well isolated colonies over about half the plate. The confluent growth becomes spotted with opaque colonies which jut above the surface of the growth, and some of these become blue and exhibit the same rapidity of growth shown by the blue daughter colonies of the isolated colonies.

If the methylene blue-eosin-xylose plate is inoculated with the slowly fermenting B. typhosus, the same phenomenon is observed. The colonies are all pink after 24 hours' incubation; a day or so later daughter colonies develop which appear by transmitted light as white dots within the pink colonies. Some of the daughter colonies soon become black. The small round black dots within the pink colony give a beautiful picture and show quite plainly that the acid production begins within the daughter colony. Some of the black daughter colonies exhibit the same rapidity of growth in the succeeding days

that was described in the case of the blue daughter colonies of the china blue plate.

If one of the large, opaque, blue colonies of the china blue-xylose plate or one of the large, black colonies of the methylene blue-eosin xylose plate is fished and inoculated into xylose broth, the latter may show acid production in 24 hours, whereas the culture inoculated on the plate may have required 6, 8, or more days to produce acid in xylose broth; if at the same time one of the small colonies or pink colonies is fished from the xylose plate into xylose broth, as much time may be required for acid production as in the case of the original culture or even more.

All of the slowly fermenting cultures examined by us have produced daughter colonies on the xylose agar plates. The number of blue colonies found on a series of china blue-xylose plates on the eighth day after inoculation with slowly fermenting cultures was:

TABLE 2

NUMBER OF BLUE COLONIES ON THE EIGHTH DAY

| Culture | |
|---|---|
| Jones | 2 |
| Wright | 25 |
| 59 | 25 |
| 75 | 5 |
| 156 | 12 |
| Rawlings | 5 |
| K-9 | 0 |
| 49 | 4 |

The cultures were inoculated at the same time on plates poured from the same batch of culture medium.

That different slowly fermenting cultures require varying lengths of time for the development of blue colonies on xylose-china blue plates is shown also by the following results:

TABLE 3

LENGTH OF TIME REQUIRED BY THE VARIOUS CULTURES FOR DEVELOPMENT OF BLUE COLONIES

| Culture | Day of Reading | Number of Blue Colonies |
|---|---|---|
| C-49 | 2 | 4 |
| C-50 | 13 | 0 |
| C-51 | 3 | 0 |
| C-51 | 5 | 8 |
| C-59 | 3 | 9 |
| C-60 | 6 | 2 |
| C-60 | 9 | 8 |
| C-175 | 6 | 2 |
| C-183 | 5 | 1 |
| C-188 | 13 | 0 |
| C-189 | 5 | 1 |
| C-48 | 9 | 11 |

Since the slowly fermenting strains of B. typhosus usually produce more and more blue colonies on the china blue-xylose plate as the period of incubation becomes longer, it was thought probable that the proportion of typhoid bacilli capable of fermenting xylose rapidly would increase in xylose broth cultures with increased time of incubation. In order to study the changes occurring in the xylose-broth culture with regard to the behavior of the contained bacilli toward xylose, it was decided to inoculate, at intervals of one or two days, one loopful of the xylose-broth culture on a xylose-china blue plate and to record the behavior of the colonies developing on these plates. Four cultures, known to ferment xylose-broth slowly, were selected for this experiment. To insure the absence of all contaminating bacteria, each culture was plated on the surface of a plain nutrient agar plate; one colony was fished from this plate, emulsified in sterile salt solution and plated on a second plain agar plate; a colony from the second plate was inoculated in like manner on a third plate; finally, a colony from the third plate was fished and inoculated on an agar slant. These agar slant cultures were used for the experiment, a xylose-broth tube being inoculated from each culture. A loopful of each of these xylose-broth cultures was inoculated at intervals on china blue-xylose plates with the results recorded in tables 4, 5, 6 and 7.

TABLE 4

CULTURE 57; THE XYLOSE-BROTH BECAME ACID IN FORTY-EIGHT HOURS

| Age of Xylose Broth Culture in Days | Days after Inoculation of the China-Blue-Xylose Plates | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 7 | 9 |
| At once | All white | All white* | 5 blue* | 7 blue | 10 blue | 22 blue | 32 blue |
| 1 | All white | 1/5 blue | | | | | |
| 2 | All pale blue | All blue | | | | | |
| 3 | Two white others blue | 4 pale blue others blue | | | | | |
| 6 | All blue | | | | | | |

* White = white colonies. * Blue = blue colonies.

Culture 57 of table 4 fermented xylose more quickly than the other cultures, producing acid in xylose in 48 hours. On the control china blue plate of the first day only 10 blue colonies had developed on

TABLE 5

CULTURE K-9. THE XYLOSE BROTH CULTURE BECAME ACID ON THE EIGHTH DAY

| Age of Xylose Broth Culture in Days | Days after Inoculation of the China-Blue-Xylose Plates | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 | 11 |
| At once | All white | All white | All white | All white | All white | 3 blue |
| 3 | | | | | 1 blue | |
| 6 | | 3 blue | | 7 blue | | |
| 8 | ½ white<br>½ blue | ⅓ white<br>⅔ blue | All blue | | | |
| 10 | All blue | | | | | |

TABLE 6

CULTURE JONES; THE XYLOSE-BROTH BECAME ACID ON THE 11TH DAY

| Age of Xylose Broth Culture in Days | Days after Inoculation of the China-Blue-Xylose Plates | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 8 | 10 | 11 |
| At once | All white | All white | All white | All white | All white | All white | All white | 4 blue |
| 1 | | | | | 1 blue | 3 blue | 7 blue | |
| 3 | | | | | 1 blue | 3 blue | | |
| 6 | | 45 blue | | ⅔ blue | | | | |
| 8 | | 6 blue | 40 blue | | | | | |
| 9 | Few white, others blue | | | | | | | |
| 12 | | | 1 white, others blue | | | | | |

TABLE 7

CULTURE RAWLINGS; THE XYLOSE BROTH BECAME ACID ON THE 8TH DAY

| Age of Xylose Broth Culture in Days | Days after Inoculation of the China-Blue-Xylose Plates | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 5 | 6 | 7 | 8 | 9 | 10 |
| At once | All white | All white | All white | All white | 3 blue | | 7 blue | |
| 1 | | | | | | 3 blue | | 12 blue |
| 3 | | | | 2 blue | | 6 blue | 7 blue | |
| 4 | | 2 blue | 6 blue | | 18 blue | 25 blue | | |
| 6 | | ¾ blue | | All blue | | | | |
| 8 | Few pale blue, many blue | All blue | | | | | | |
| 10 | All blue | | | | | | | |

the fifth day. After the bacteria had grown for only 24 hours in the xylose broth, they had undergone a marked change; for, when inoculated on a china blue xylose plate, about one-fifth of all the colonies on the plate were blue on the second day. Culture K-9 of table 2 had developed on the control plate only 3 blue colonies on the eleventh day. After 7 days' growth in the xylose broth the bacteria had changed only slightly in their behavior toward xylose, but during the next 2 days they underwent a profound change, most of the bacteria producing blue colonies on the xylose plate quite promptly Cultures Jones and Rawlings behaved similarly to K-9 but the change in the bacteria apparently took place somewhat more gradually.

To prove that the development of the daughter colonies in the preceding experiments was in no wise due to the stains contained in the agar, slowly fermenting cultures were inoculated on plain nutrient agar containing 1% xylose; on these plates daughter colonies developed in the same manner as on the china blue-xylose plates and the methylene blue-eosin xylose plates. The great majority of the cultures tested showed no daughter colonies on the control agar plates without xylose; the others showed very small daughter colonies which developed very late—usually after the tenth day of incubation. Cultures that fermented xylose rapidly, as well as those that fermented this sugar slowly, were represented among the cultures showing the late development of small daughter colonies on the control plates; among these a rapidly fermenting culture, obtained through the kindness of Dr. A. F. Coca from the New York Hospital soon after isolation from a blood culture, revealed the best developed daughter colonies on the control plate. Since the daughter colonies due to the presence in the medium of xylose appeared between the second and fifth days of incubation, the late daughter colonies, probably called forth by some unknown substance in the nutrient agar, caused no confusion. We gained the impression that daughter colonies were less likely to appear on the control plates if the cultures had been carried for a long time on nutrient agar than if they had been isolated from patients comparatively recently.

A systematic attempt was made to obtain from each of the cultures that fermented xylose slowly a subculture which would ferment this

sugar in 24 hours. Two methods were employed to accomplish this purpose: First, transplants were made from the xylose broth cultures after a number of days' incubation to fresh xylose broth tubes; and second, the cultures were inoculated on china blue-xylose plates and large blue colonies, which developed on the plates, were fished and inoculated into xylose broth. Rapidly fermenting subcultures were readily obtained from many of the cultures by both of these methods. Some of the cultures, however, were quite refractory and positive results were obtained in these instances only after repeated attempts. Rapidly fermenting subcultures have been obtained from all of the slowly fermenting strains with the single exception of culture C-188. These subcultures were plated to demonstrate their purity and were agglutinated with a typhoid immune serum and found to agglutinate to the same limits as the original cultures. Since cultures C-188 and C-189 were from the same patient, the former from a blood culture, the latter from a stool, they may be regarded as the same strain; we succeeded in obtaining from C-189 a subculture that fermented xylose in 24 hours.

*Arabinose Broth.*—It is generally stated that B. typhosus does not ferment arabinose. Nevertheless, we carried out a series of experiments with this sugar similar to those described above for xylose. It will suffice for the present to indicate briefly the results obtained, the discussion of these results being reserved for a later section of this articles.

June 2, 1919, in the first series of tests, 114 cultures of groups 1 and 2 were employed. The medium consisted of meat infusion rendered sugar-free by inoculation with B. coli, to which was added 1% peptone, 0.5% sodium chlorid and 1% arabinose. Litmus was the indicator. Sterilization was carried out in the autoclave at 10 lbs.' pressure for 10 minutes.

| | |
|---|---|
| Positive on the 2nd day (1) | 1 culture |
| Positive on the 7th day (9, 34, 134 and 138) | 4 cultures |
| Positive on the 9th day (63) | 1 culture |
| Positive on the 11th day (16) | 1 culture |
| Positive on the 28th day (3) | 1 culture |
| Negative on the 28th day | 107 cultures |

Only 7% of the cultures showed acid production in the arabinose broth tubes of this series.

June 22, 1919, in the second series of tests, 117 cultures of groups 1 and 2 were employed. The medium contained 1% peptone, 0.25% nutrose and 1% arabinose. Phenol red and decolorized china blue served as indicators.

| | |
|---|---|
| Positive on the 3rd day (75) | 1 culture |
| Positive on the 4th day (100 and 161) | 2 cultures |
| Positive on the 10th day (63) | 1 culture |
| Positive on the 14th day (31) | 1 culture |
| Positive on the 23rd day (162) | 1 culture |
| Positive on the 24th day (28) | 1 culture |
| Negative on the 30th day | 110 cultures |

Only 6% of the cultures showed acid production in the arabinose broth. Although there is a fairly good agreement in the percentage of positives in the two sets of tests, yet the positives of the first series were with one exception, culture 63, negative in the second series.

June 26, 1919, in the third series of tests, the 12 cultures from France that fermented xylose slowly and 9 recently isolated cultures were used. The medium was the same as in the second series.

| | |
|---|---|
| Positive on the 6th day (C-188) | 1 culture |
| Negative on the 32nd day | 20 cultures |

July 4, 1919, in the fourth series of tests, the cultures that had given positive results in either of the first two series of tests and the 12 cultures from France that fermented xylose slowly were plated on plain nutrient agar, and a single colony from each plate was fished and inoculated on an agar slant. The latter cultures were employed in this experiment. The medium was meat infusion rendered sugar-free by inoculation with B. coli, to which was added 1% peptone, 0.5% sodium chlorid and 1% arabinose. Phenol red and decolorized china blue served as indicators.

| | |
|---|---|
| Positive on the 2nd day (98) | 1 culture |
| Positive on the 6th day (C-51, C-59, C-175, 191 and 134) | 5 cultures |
| Positive on the 10th day (C-176) | 1 culture |
| Negative on the 25th day | 20 cultures |

*Arabinose Plates.*—All of the typhoid cultures inoculated by us on arabinose-china blue plates have without exception produced daughter colonies similar to those described above. In the case of xylose we have seen that only about 10% of the cultures, namely, those that fermented xylose in broth very slowly, gave rise to daughter colonies. On the arabinose china-blue plates each isolated colony usually contains many small daughter colonies; between the second and tenth days, one or two or more blue colonies may appear. A number of the latter, when fished into arabinose broth, caused acid production in 24 hours. The blue colonies grow with great rapidity, but, instead of yielding a heaped up growth as in the case of xylose, they usually give a flat spreading growth which may form an opaque, round colony having several times the diameter of the original colony from which it started. Furthermore, the large blue colonies on the arabinose plate do not usually increase in number on further incubation to the same extent as is the case with the slowly fermenting typhoid cultures on xylose plates.

We have made no systematic attempt to obtain from each typhoid culture of our collection a subculture that would ferment arabinose in 24 hours, but we have obtained a number of such subcultures and have proved them to be typhoid bacilli by means of the agglutination test. These subcultures when inoculated on arabinose-china blue plates yield colonies, uniform in type, which do not develop daughter colonies. Our records show that we have inoculated on china blue-arabinose plates 33 different typhoid cultures, 11 of which were of the type that

ferments xylose rapidly and 22 of the type that ferments xylose slowly; 5 of the former group and 8 of the latter group gave rise to one or more large blue colonies. In the arabinose-broth tests also positive cultures were found among the strains that fermented xylose slowly, as well as among those that fermented xylose rapidly. There thus appears to be no relation between the development of ferments for xylose and for arabinose by different strains of B. typhosus, although these two sugars are closely related chemically.

When subcultures that fermented xylose rapidly were obtained from slow fermenters, these subcultures remained rapid fermenters when kept on agar slants containing no xylose. By fishing both blue and white colonies from xylose-china blue plates on agar slants and at the same time into xylose broth, we obtained rapid and slow fermenters from the same cultures. Twenty-five of these subcultures, which gave a good acid reaction in xylose broth in from 24 to 48 hours, yielded the same result when inoculated into xylose broth from 1 to 3 months later, though they had not been in contact with xylose during the interval. Eighteen subcultures that produced acid not at all or after the eighth day on the first test reacted in the same way after from 1 to 3 months on plain agar. One subculture, 57-C-ii, which produced acid only after 20 days on the first test, gave an acid reaction on the 7th day in the second test. Two subcultures, C-189-1 and Lewis B-2, which were positive on the third and fifth day, respectively, in the first test, were negative on the sixteenth day of the second test.

Similarly, 19 subcultures that fermented arabinose in from 24 to 48 hours on the first test still fermented arabinose after an interval of from 1 to 2 months on plain agar, in from 24 to 48 hours. Since most of the typhoid cultures gave negative results in arabinose broth, only a few of the white colonies were fished from arabinose-china blue plates; five of these were negative in both tests, one was negative in the first test and gave acid reaction in arabinose broth on the fourth day in the second test.

All of these subcultures from the arabinose and xylose plates were shown to be typhoid bacilli by agglutination with a typhoid serum.

*Dulcite Broth.*—It will be seen below that none of the cultures tested produced acid in dulcite broth before the fourth day. A large percentage of the cultures — in the first series, 37 — fermented the xylose if the tubes were observed for a period of 30 days. The positive cultures of one series of tests did not at all correspond with the

positives of other series; a culture might ferment the dulcite comparatively early in one test and not produce an acid reaction at all in two or three succeeding tests. We have seen in the foregoing that many typhoid cultures behaved in a similar way with regard to arabinose broth, but that the two groups of cultures were quite constant in their behavior in xylose broth in that the rapid fermenters always fermented rapidly and the slow fermenters never fermented rapidly, though the time required by the latter in different tests varied considerably.

June 2, 1919, in the first series of tests, 115 typhoid cultures of groups 1 and 2 were used. The medium consisted of meat infusion, rendered free from sugar by inoculation with B. coli, to which were added 1% peptone, 0.5% sodium chlorid and 1% dulcite. Litmus was the indicator.

| | |
|---|---|
| Positive on the 4th day | 1 culture |
| Positive on the 7th day | 15 cultures |
| Positive on the 9th day | 8 cultures |
| Positive on the 11th day | 7 cultures |
| Positive on the 13th day | 9 cultures |
| Positive on the 16th day | 2 cultures |
| Positive on the 19th day | 1 culture |
| Negative on the 40th day | 72 cultures |

Forty-three of the cultures, or 37%, were positive.

June 22, 1919, in the second series of tests, 57 cultures of group 1 were used. The dulcite broth was prepared as in the preceding tests.

| | |
|---|---|
| Positive on the 10th day | 1 culture |
| Positive on the 14th day | 5 cultures |
| Positive on the 23rd day | 12 cultures |
| Positive on the 30th day | 9 cultures |
| Negative on the 30th day | 30 cultures |

Twenty-seven of the cultures, or 47%, were positive.

June 26, 1919, in the third series, 9 recently isolated cultures, all except one of which fermented xylose rapidly, and 12 cultures from France, which fermented xylose slowly. The medium contained 0.25% nutrose, 0.5% sodium chlorid, 1% peptone and 1% dulcite. Phenol red and decolorized china blue served as indicators.

| | |
|---|---|
| Positive on the 14th day | 6 cultures |
| Positive on the 16th day | 1 culture |
| Negative on the 32nd day | 14 cultures |

Four of the strains that ferment xylose rapidly were positive and only 3 of the 12 cultures that ferment xylose slowly were positive.

July 4, 1919, in the fourth series, 12 cultures from France which ferment xylose slowly and 17 selected cultures, which in at least one test had fermented arabinose in broth, were used. The medium consisted of meat infusion, rendered sugar-free by inoculation with B. coli, to which were added 1% peptone, 0.5% sodium chlorid and 1% dulcite. The cultures were plated on nutrient agar and single colonies were fished and inoculated on agar slants; the latter cultures were used in the experiment.

| | |
|---|---|
| Positive on the 10th day | 1 culture |
| Positive on the 15th day | 2 cultures |
| Positive on the 31st day | 1 culture |
| Negative on the 31st day | 25 cultures |

Only 14% of the cultures were positive; the smaller percentage of positives in this test than in the preceding tests may have been due to the fact that cultures freshly started from a single colony were used.

A fifth series, including all of our 139 cultures, gave 74 positives on or before the twenty-sixth day; that is, 54% of positives.

In all of the above dulcite-broth tests there was a greater tendency for the indicator (litmus or china blue) to become reduced than in the tests with xylose broth or arabinose broth; it is highly probable that some of the tubes that were recorded as negative were, in fact, positive, and that the percentage of positives, as given, is consequently too low.

*Dulcite plates.*—When typhoid cultures are inoculated on dulcite-china blue plates, daughter colonies appear on from the second to fifth day. They appear at first in comparatively few of the colonies on the plate and each of these colonies usually contains only one or two daughter colonies; in both of these respects the dulcite plate is different from the xylose or arabinose plates. Another difference consists in the fact that almost all the daughter colonies on the dulcite plate increase rapidly in size, while on the xylose or arabinose only an extremely small percentage of the daughter colonies, which are usually crowded together within a colony, exhibit this rapid growth. It is quite characteristic for the confluent growth on the dulcite plate to become studded with large opaque colonies and large opaque daughter colonies appear also in scattered isolated colonies. Such daughter colonies, almost without exception, grow very rapidly, yielding a heaped up growth, and some of them become blue or have blue centers, though there is a tendency for the china blue to become reduced as the growth further increases. It is the rule rather than the exception for the dulcite plate to have 25 or 50 or more of the large opaque colonies between the fifth and tenth day of incubation. When the large blue colonies are fished and inoculated into dulcite broth, some produce acid in 48 hours, others produce acid more slowly, still others call forth an alkaline reaction, and many cause reduction of the indicator.

No culture that we have tested has failed to produce the large opaque colonies on the dulcite agar and we have tested both cultures that ferment xylose slowly and cultures that ferment xylose rapidly—in all 40 cultures.

These experiments have furnished some evidence that ferments for xylose, arabinose and dulcite appear quite independently of each other in typhoid cultures. It was planned to secure by artificial means strains or subcultures showing maximum and minimum activity respectively for each of the sugars and then to test strains against all three of the sugars. The method pursued was to plate a culture on a china blue plate containing the sugar and to fish from the plate, after a suitable period of incubation, a blue and a white colony. The blue colony was inoculated into broth containing the sugar in question and was transplanted in such broth at intervals, if necessary, until a strongly acid reaction was produced in 24 hours. The slowly fermenting strains were kept on agar slants until time for the experiment. The results of this experiment are recorded in table 8.

Both the subcultures that fermented xylose slowly and those that fermented xylose rapidly failed to ferment arabinose; one of the rapid xylose fermenters and one of the slow xylose fermenters produced acid in dulcite.

Three of the rapid arabinose fermenters and four of the slow (or negative) arabinose fermenters produced acid in dulcite. The subculture A-K-9-1, which was trained to ferment arabinose in 24 hours, continued to ferment xylose slowly like the culture from which it was derived. The same is true of the culture D-K-9-1, which was trained to ferment dulcite in 48 hours. The two subcultures from the culture Jones, which fermented dulcite rapidly and slowly respectively, both fermented xylose slowly, though the rapid dulcite fermenter did produce acid in xylose earlier than the slow dulcite fermenter. Two of the rapid dulcite fermenters produced acid in arabinose, while none of the slow dulcite fermenters did so.

RESULT IN XYLOSE, ARABINOSE AND DULCITE BROTH

| Culture | Type | Xylose Broth | | | | | | | Arabinose Broth | | | | Dulcite Broth | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 Day | 3 Days | 4 Days | 6 Days | 9 Days | 21 Days | 35 Days | 1 Day | 4 Days | 21 Days | 35 Days | 1 Day | 2 Days | 3 Days | 4 Days | 6 Days | 9 Days | 17 Days | 27 Days | 35 Days |
| Rawling I | Rapid in xylose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| Rawlings II | Slow in xylose | ± | .. | .. | .. | .. | .. | — | ± | .. | .. | — | ± | .. | .. | .. | .. | ++ | .. | .. | ++ |
| Jones M I | Rapid in xylose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| Jones M II | Slow in xylose | ± | .. | .. | .. | .. | .. | — | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| 156 I | Rapid in xylose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| 156 II a | Slow in xylose | ± | .. | .. | .. | .. | .. | — | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| 57 C I | Rapid in xylose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| 57 C II | Slow in xylose | ± | ++ | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| K-9 II C II | Rapid in xylose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | ++ | .. | .. | .. | .. | .. | ++ |
| K-9 II C I | Slow in xylose | ± | .. | .. | .. | .. | .. | — | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| Wright P II | Rapid in xylose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| Wright C II a | Slow in xylose | ± | .. | .. | .. | ++ | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| A 18 I | Rapid in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ++ | .. | .. | ++ | ± | .. | .. | .. | .. | .. | .. | .. | — |
| A K-9 I | Rapid in arabinose | ± | .. | .. | ++ | .. | .. | ++ | ++ | .. | .. | ++ | ± | .. | .. | .. | .. | ++ | .. | .. | ++ |
| A 9 | Rapid in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ++ | .. | .. | ++ | ± | .. | .. | .. | ++ | .. | .. | .. | .. |
| A 32 | Rapid in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ++ | .. | .. | ++ | ± | .. | .. | .. | .. | .. | .. | .. | — |
| A 34 | Rapid in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ++ | .. | .. | ++ | ± | .. | .. | .. | .. | .. | .. | .. | — |
| A 51 | Rapid in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ++ | .. | .. | ++ | ± | .. | .. | .. | .. | .. | ++ | .. | ++ |
| A 134 | Rapid in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ++ | .. | .. | ++ | ± | .. | .. | .. | .. | .. | .. | .. | — |
| a 2 | Slow in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| a 25 | Slow in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| a 78 | Slow in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | ++ | .. | .. | ++ |
| a 91 | Slow in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | ++ | .. | .. | ++ |
| a 105 | Slow in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | ++ | .. | ++ |
| a 163 D | Slow in arabinose | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | ++ | .. | .. | .. | .. | ++ |
| D Jones II | Rapid in dulcite | ± | .. | ++ | .. | .. | .. | .. | ± | .. | .. | — | ++ | .. | .. | .. | .. | .. | .. | .. | ++ |
| D K-9 I | Rapid in dulcite | ± | .. | .. | .. | .. | ++ | ++ | ± | .. | .. | — | .. | ++ | .. | .. | .. | .. | .. | .. | ++ |
| D 2-I | Rapid in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ++ | .. | .. | .. | .. | .. | .. | .. | ++ |
| D K31-I | Rapid in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ++ | .. | .. | .. | .. | .. | .. | .. | ++ |
| D 163 D I | Rapid in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ++ | .. | .. | .. | .. | .. | .. | .. | ++ |
| D 126 | Rapid in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | ++ | ++ | ++ | .. | .. | .. | .. | .. | .. | .. | ++ |
| D 129 | Rapid in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ++ | .. | .. | .. | .. | .. | .. | .. | — |
| D 136 | Rapid in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | ++ | .. | ++ | ++ | .. | .. | .. | .. | .. | .. | .. | ++ |
| D 163 I | Rapid in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ++ | .. | .. | .. | .. | .. | .. | .. | ++ |
| d 163 II | Slow in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| d 3 | Slow in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| d 106 | Slow in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | ++ |
| d 127 | Slow in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| d 136 | Slow in dulcite | ++ | .. | .. | .. | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |
| d Rawling | Slow in dulcite | ± | .. | .. | ++ | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | ++ | ++ |
| d Jones | Slow in dulcite | ± | .. | .. | ++ | .. | .. | ++ | ± | .. | .. | — | ± | .. | .. | .. | .. | .. | .. | .. | — |

All in all, this experiment offers strong evidence that an increased or decreased production of the ferment for one of the three sugars — xylose, arabinose, or dulcite — induced by experimental means in a typhoid culture, does not affect the production by the culture of ferments for the other sugars.

Three subcultures that fermented xylose rapidly were transplanted every 3 days in xylose broth for 2 weeks; 3 rapid arabinose cultures were similarly transplanted in arabinose broth, and 3 rapid dulcite fermenters in dulcite broth. These 9 cultures were also transplanted every 3 days for 2 weeks in sugar-free broth. At the end of the time both sets of cultures were inoculated into xylose broth, arabinose broth and dulcite broth, as were also 9 slowly fermenting cultures. The results of this experiment are recorded in tables 9, 10 and 11.

TABLE 9

RAPIDLY FERMENTING CULTURES TRANSPLANTED EVERY THREE DAYS FOR TWO WEEKS IN BROTH CONTAINING THE SUGAR THAT IS FERMENTED

| Culture | Transplanted in | Xylose Broth | | | Arabinose Broth | | | Dulcite Broth | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 Day | 6 Days | 28 Days | 1 Day | 4 Days | 28 Days | 1 Day | 4 Days | 11 Days | 19 aDys | 28 Days |
| X Rawling I | Xylose broth | ++ | .. | ++ | ± | .. | — | .. | .. | ++ | .. | .. |
| X Jones M I | Xylose broth | ++ | .. | ++ | ± | .. | — | .. | ++ | .. | .. | ++ |
| X K 9 II C II | Xylose broth | ++ | .. | ++ | ± | .. | — | .. | ++ | .. | .. | .. |
| A K 9-1 | Arabinose broth | ± | .. | — | ++ | .. | ++ | .. | .. | .. | ++ | ++ |
| A 9 M | Arabinose broth | ++ | .. | ++ | ++ | .. | ++ | ± | .. | .. | .. | — |
| A 32 M | Arabinose broth | ++ | .. | ++ | ++ | .. | ++ | ± | .. | .. | .. | — |
| D Jones I | Dulcite broth | .. | ++ | ++ | ± | .. | — | ++ | .. | .. | .. | ++ |
| D 163 I | Dulcite broth | ++ | .. | ++ | .. | ++ | .. | ++ | .. | .. | .. | ++ |
| D 126 | Dulcite broth | ++ | .. | ++ | ± | .. | — | ++ | .. | .. | .. | ++ |

TABLE 10

THE SAME RAPIDLY FERMENTING CULTURES TRANSPLANTED EVERY THREE DAYS FOR TWO WEEKS IN SUGAR-FREE BROTH

| Culture | Transplanted in | Xylose Broth | | Arabinose Broth | | Dulcite Broth | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 Day | 28 Days | 1 Day | 28 Days | 1 Day | 3 Days | 9 Days | 28 Days |
| X Rawling I | Xylose broth | ++ | ++ | ± | — | .. | ++ | .. | .. |
| X Jones M I | Xylose broth | ++ | ++ | ± | — | .. | ++ | .. | .. |
| X K 9-II C II | Xylose broth | ++ | ++ | ± | — | ± | .. | .. | — |
| A K 9-1 | Arabinose broth | ± | — | ++ | ++ | .. | .. | ++ | .. |
| A 9 M | Arabinose broth | ++ | ++ | ++ | ++ | ± | .. | .. | — |
| A 32 M | Arabinose broth | ++ | ++ | ++ | ++ | ± | .. | .. | — |
| D Jones I | Dulcite broth | ± | — | ± | — | ++ | .. | .. | ++ |
| D 163 I | Dulcite broth | ++ | ++ | ± | — | ++ | .. | .. | ++ |
| D 126 | Dulcite broth | ++ | ++ | ± | — | ++ | .. | .. | ++ |

TABLE 11

SLOWLY FERMENTING CULTURES

| Culture | Type | Xylose Broth | | | Arabinose Broth | | | Dulcite Broth | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 Day | 19 Days | 28 Days | 1 Day | 6 Days | 28 Days | 2 Days | 6 Days | 9 Days | 11 Days | 12 Days | 28 Days |
| x K 9-11 C I | Slow in xylose | ± | .. | — | ± | .. | — | — | ++ | .. | .. | .. | ++ |
| x Jones M II | Slow in xylose | .. | ++ | ++ | ± | .. | — | ++ | ++ | .. | .. | .. | ++ |
| x Rawlings II a | Slow in xylose | ± | .. | — | ± | .. | — | .. | .. | ++ | .. | .. | .. |
| a 105 | Slow in arabinose | ++ | .. | ++ | ± | ++ | ++ | .. | .. | ++ | .. | .. | .. |
| a 91 | Slow in arabinose | ++ | .. | ++ | .. | .. | .. | ± | .. | .. | .. | .. | — |
| a 78 | Slow in arabinose | ++ | .. | ++ | ± | ++ | ++ | .. | ++ | .. | .. | .. | ++ |
| d 3 | Slow in dulcite | ++ | .. | ++ | ± | .. | — | .. | .. | .. | .. | ++ | .. |
| d 163 II | Slow in dulcite | ++ | .. | ++ | ± | ++ | .. | ± | .. | .. | .. | .. | — |
| d Jones | Slow in dulcite | ± | .. | — | ± | .. | — | ± | .. | .. | ++ | .. | .. |

It is seen that the further cultivation of a rapidly fermenting strain in broth containing the sugar that it fermented rapidly did not influence the production of ferments for the other two sugars. It is seen also that the removal of the rapid fermenters from contact with the sugars for a period of two weeks, during which time they were transplanted every three days, leads to no apparent diminution in the rapidity with which they fermented their respecttive sugars.

Inoculation on china blue agar containing a given sugar often affords a more accurate idea of the behavior of a culture toward this sugar than does inoculation into broth containing the sugar. Consequently, 3 rapid fermenters and 1 slow fermenter of each of the 3 sugars were inoculated on a series of china blue plates containing 1% of xylose, arabinose, dulcite and rhamnose. Rhamnose was also employed because all the typhoid cultures inoculated on rhamnose agar had been found by us to yield many daughter colonies. Cultures which fermented rapidly the sugar contained in the agar produced blue colonies and no daughter colonies appeared on these plates. All of the cultures — the rapid fermenters of xylose, arabinose and dulcite as well as the slow fermenters — produced daughter colonies on the rhamnose plates. Subcultures of slow xylose fermenters, which had been trained to ferment arabinose or dulcite rapidly, behaved on the xylose plates like the original slow xylose fermenters from which they were derived. Rapid arabinose fermenters and rapid xylose fermenters produced the usual daughter colonies and large opaque colonies

on dulcite plates. Thus additional evidence was obtained in favor of the view that the production of the ferment for one of these sugars may be increased without affecting the ferments for the other two sugars.

It was decided to inoculate typhoid cultures on a number of sugars and on salicin and glycerol to see if other sugars besides those investigated above gave rise to daughter colonies Six cultures that were known to ferment xylose slowly and two that fermented xylose rapidly were selected for the experiment; one of the rapid fermenters had been recently isolated from the blood of a typhoid patient and showed many daughter colonies on the control plate on the fourteenth day. All of the sugars and salicin were used in 1% amounts, but 3% glycerol was employed. None of the sugars except xylose, arabinose and dulcite gave rise to daughter colonies, but the types of colonies that developed on the other plates after a few days' incubation were interesting. On the dextrose, mannite, maltose and galactose plates the colonies were small, round, flat and opaque, and apparently increased but little in size after the first 48 hours' incubation. On the salicin, lactose, saccharose and raffinose plates the colonies were like those of the control plates, while on dextrin they appeared to be somewhat larger and thicker than on the control plates. On the glycerol plates the colonies soon became very much larger and thicker than the colonies of the control plates, and after five or six or more days of incubation they were quite different from the colonies on all the other plates, so that the glycerol plates could be picked from the others at a glance; still later, the colonies on the glycerol plates became quite brown. Rhamnose was not available to us at the time this experiment was performed.

The colonies in which daughter colonies develop on the xylose, arabinose and rhamnose plates are transparent in type, being usually somewhat thinner and more delicate than the typhoid colonies on the control plate.

With regard to the smallest amounts of the sugars that give rise to daughter colonies, we have found that cultures giving good daughter colonies on 1% xylose agar on the second or third day require from 10 to 12 days on 0.2% xylose agar, and produce very few daughter colonies on 0.04% of xylose. On 0.25% and 0.1% arabinose the daughter colonies are smaller and appear later than on 1% arabinose agar. Rhamnose yields well developed daughter colonies in much greater dilution than xylose and arabinose, 0.02% of rhamnose giving good daughter colonies on the 4th day, and 0.01% many small daughter colonies on the 10th day. One one hundreth per cent. of dulcite also gives rise to daughter colonies.

One fourth per cent. dextrose added to 1% xylose agar did not interfere with the production of daughter colonies. No daughter colonies were observed on plates containing 1% xylose and 0.5 or 2% glycerin.

*Raffinose.*—None of our typhoid cultures produced acid in raffinose broth; after 30 days' incubation all of the tubes showed an alkaline reaction. No daughter colonies appeared on raffinose agar plates. Three per cent. and 2% raffinose agar gave rise to typhoid colonies that were larger and more opaque than the colonies of the control plate, thus indicating that B. typhosus is able to utilize this sugar as a food.

*Inosite.*—None of our typhoid cultures produced acid in inosite broth. No daughter colonies were seen on the inosite plates. Three per cent. inosite does not inhibit the growth of B. typhosus.

## DISCUSSION

Having presented the results of our experiments in some detail, we shall next consider the results of similar experiments that have been carried out by other workers. Daughter colonies have been observed and studied in connection with cultures of B. anthracis, V. cholerae, B. coli, B. dysenteriae and some other organisms, but they were either caused by sugars not considered in this paper or were not due to sugars at all. Twort,[5] after growing a strain of B. typhosus for two years in lactose medium, succeeded in producing a strain that fermented lactose. This experience is unique; Penfold,[6] working with 20 strains and having carried many of them for more than a year in lactose medium, obtained only negative results; he showed that the Twort lactose fermenting strain produced daughter colonies on lactose agar. This Twort culture fermented sorbite in broth only after a number of days, and Penfold found that it also gave rise to daughter colonies on sorbite agar.

Reiner Mueller[7] showed that B. typhosus produces daughter colonies on rhamnose agar. He examined a large number of cultures in this regard and found that they all gave rise to daughter colonies. He did not see acid production in rhamnose by any of his typhoid cultures. He noticed that the colonies on the rhamnose plates remain small and delicate and found that 5% rhamnose produced no more inhibition than 0.5% in the agar. He showed further that other bacteria of the typhoid-coli group are not inhibited by rhamnose and that B. typhosus gives rise to daughter colonies in 8 days on agar containing as little as 0.025% of rhamnose and in 14 days on agar containing only 0.01%. He endeavors to bring the inhibition into causal connection with the production of daughter colonies by assuming that certain bacteria within the typhoid colony overcome the inhibition, grow rapidly and give rise to daughter colonies. Penfold found that the 20 strains of B. typhosus investigated by him all gave daughter colonies on rhamnose neutral red agar and he noticed acid production in none of the daughter colonies. He did observe with some of his cultures late acid production in rhamnose broth and, on transplanting from rhamnose broth to rhamnose broth after several weeks of incubation, he was able to obtain subcultures which fermented in 1, 2 or 3 days. Such a rapid fermenter no longer produced daughter colonies on rhamnose agar and, even when it was passed through 13 generations of peptone water and plated on rhamnose agar, it still did not give rise to

[5] Proc. Roy. Soc., London, 1907, 79, p. 329.

[6] Jour. of Hyg., 1911, 11, p. 30.

[7] Centralbl. f. Bakteriol., I., O., 1911, 58, p. 97.

daughter colonies. He found that the Twort lactose-fermenting B. typhosus and a typhoid culture which had been trained to ferment dulcite rapidly both produced daughter colonies on rhamnose agar.

Reiner Mueller suggests that the development of daughter colonies on rhamnose agar might be utilized in the identification of B. typhosus; the results obtained by Penfold and by us, as far as they go, indicate that he was right in concluding that all typhoid cultures exhibit the phenomenon. Mueller found that some other bacteria besides B. typhosus also give rise to daughter colonies on rhamnose agar.

Typhoid bacilli inoculated on litmus agar containing arabinose, dulcite or raffinose produced no change in the medium according to Reiner Mueller. This is difficult to understand in view of Penfold's results with dulcite and our results with arabinose and dulcite. Penfold made a very careful study of the behavior of B. typhosus in dulcite broth and on neutral red dulcite agar. In one of his experiments in which 14 strains were inoculated into dulcite broth, the first signs of acidity occurred in from 5 to 15 days. If, after one month, subcultures were made in new dulcite broth, an acid reaction was produced in from 1 to 4 days. Three strains inoculated on neutral red dulcite agar yielded daughter colonies as early as the 3rd day and some of the latter were acid by the 5th day. Some plates showed as low as 2% of colonies with daughter colonies, some as high as 50%. Different plates inoculated with the same culture also showed variations within these limits. Subcultures, which had been trained to ferment dulcite rapidly, showed great permanency; one such culture, transplanted 25 times in peptone water during a period of 5 months and then plated on neutral red dulcite agar, yielded only fermenting colonies. Twenty colonies from a MacConkey plate of pure typhoid were inoculated into dulcite broth; the time required for acidity to appear varied from 11 to 32 days. This observation is in harmony with our findings with regard to the varying results yielded by typhoid cultures on successive tests in dulcite broth.

Krumwiede, Kohn and Valentine[8] inoculated 37 strains of B. typhosus into xylose broth and found that 29 produced acid in 24 hours while 8 of the strains required from 5 to 13 days for the production of acid.

Mandelbaum[9] obtained in Munich from the blood or feces of more than 50 patients with clinical typhoid fever a bacillus which he called Bacterium metatyphi. This bacillus resembled B. typhosus in all respects except that it produced alkali instead of acid in mediums containing glycerol. He showed that these cases were infected, in all probability, from a typhoid carrier, a woman who served as a milker in a dairy near Munich. The interesting observation was made that this carrier had both the typical B. typhosus and the B. metatyphi in her stools. The B. metatyphi had retained the property of producing alkali in glycerol mediums for 5½ years when transplanted on plain nutrient agar.

Some of the strains of B. metatyphi produced daughter colonies on glycerol agar, and from the daughter colonies cultures were obtained which behaved in all respects like B. typhosus. Since B. metatyphi would be overlooked in the usual technic for the isolation of B. typhosus, little is known at present concerning its prevalence. Russowici[10] reported having found one strain, and

[8] Jour. Med. Res., 1918, 38, p. 89.

[9] Centralbl. f. Bakteriol., I., O., 1912, 63, p. 46.

[10] München. med. Wchnschr., 1908, 55, p. 2507.

[11] Centralbl. f. Bakteriol., I., O., 1911, 58, p. 97.

Ditthorn and Luerssen[11] two strains. All of our 138 cultures produced acid in glycerol broth.

From a small epidemic of clinical typhoid fever in an insane asylum in Denmark Jacobsen[12] obtained a bacillus which he described as Bacterium typhi mutabile. The bacillus resembled B. typhosus in all respects except the following: (1) Its growth was strongly retarded on Konradi-Drigalski agar or plain nutrient agar, which had been sterilized in the autoclave, and (2) it did not agglutinate in typhoid immune serum. On the plates showing retarded growth, a few large colonies appeared on the 5th or 6th day, which when fished on agar slants gave typical agglutination with typhoid immune serum and resembled B. typhosus also in all other respects. B. typhi mutabile itself gave good agglutination three months after its isolation. Although there was an inhibited growth on Conradi-Drigalski plates, yet the organism exhibited good growth on Endo plates. Jacobsen showed that it was the sodium sulphite of the Endo plates which abolished the inhibition. Fromme[13] studied a bacillus the growth of which was retarded on nutrient agar but good on nutrient agar to which sodium sulphite was added. His bacillus differed from Jacobsen's in that it agglutinated with typhoid immune serum from the start and in that no large colonies developed on the plates showing the retarded growth.

It has been shown that the so-called nonfermenters of Weiss are in reality slow fermenters and can be made to revert to the typical rapidly fermenting type of B. typhosus. There are the following variants of B. typhosus:

1. Bacterium metatyphi (Mendelbaum), a nonfermenter of glycerol; in contact with glycerol it reverts to the typical B. typhosus.

2. Bacterium typhi mutabile (Jacobsen), the growth of which is inhibited on autoclaved nutrient agar; it reverts to the typical B. typhosus.

3. Nonfermenter of xylose (Weiss), really slow fermenters of xylose, which, growing in contact with xylose, revert to the typical B. typhosus.

4. Artificially produced variants (rapid arabinose fermenters, rapid dulcite fermenters and others).

Two other variants may be mentioned in passing: (a) freshly isolated strains which do not agglutinate with typhoid immune serum; they usually agglutinate typically after having been transplanted a number of times on artificial mediums, and (b) the so-called "blue typhoids" which produce a deep blue color in litmus milk early (from the third to sixth day); they retain this property indefinitely when propagated on nutrient agar.

[12] Centralbl. f. Bakteriol., I., O., 1910, 56, p. 208.
[13] Centralbl. f. Bakteriol., I., O., 1911, 58, p. 445.

It is obvious from the discussion that B. typhosus should not be divided into different types or groups or varieties. The variants described are very interesting and may at times, as suggested by Lieut. R. C. Colwell, be of epidemiologic value in tracing the source of infections, but since they revert to the typical B. typhosus under certain conditions, and since they have not been shown to be constantly and permanently different from B. typhosus serologically, they cannot be allowed to alter the conception of B. typhosus as a homogeneous organism.

## SUMMARY

The so-called nonfermenters of xylose among typhoid strains were shown to be slow fermenters; rapidly fermenting subcultures were obtained from them.

The slowly fermenting strains produce daughter colonies on 1% xylose agar plates; some of these daughter colonies increase greatly in size and produce acid on further incubation.

Only a small percentage of the typhoid cultures investigated produced acid in arabinose broth, and these cultures did not do so constantly. Rapidly fermenting subcultures were obtained from a number of strains by plating on arabinose-china blue agar.

Typhoid cultures inoculated on 1% arabinose agar gave rise to daughter colonies; a few of the daughter colonies may increase greatly in size and produce acid on further incubation.

Subcultures that ferment arabinose rapidly still retain this characteristic after having been kept on plain nutrient agar for one or two months; the same is true of the subcultures that ferment xylose rapidly, which were obtained from slowly fermenting strains.

The amount of ferment for one of the sugars, xylose, arabinose or dulcite produced by a strain of B. typhosus may be greatly increased without affecting the production of ferments for the other two sugars.

The evidence at hand, it is thought, does not justify the division of typhoid bacilli into separate groups.

## EXPLANATION OF PLATE

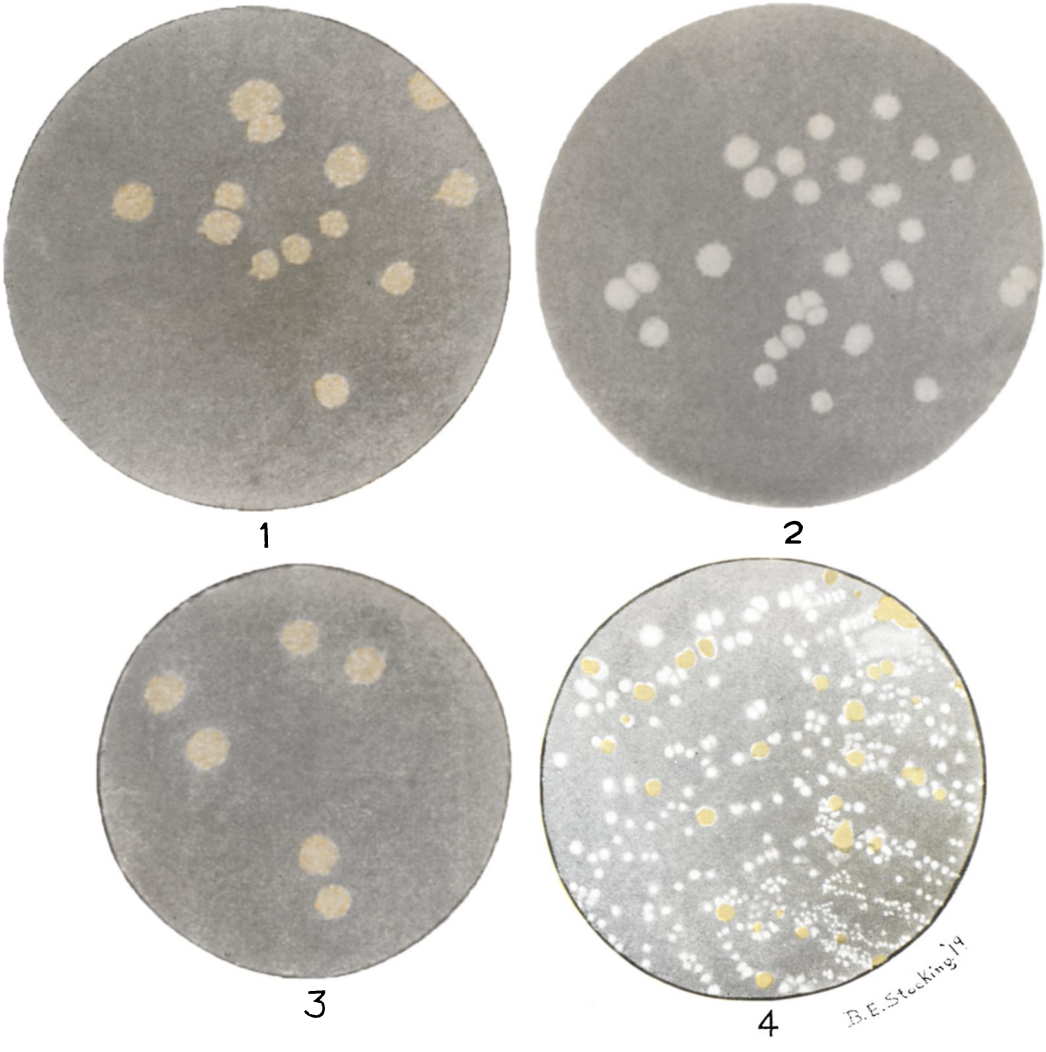
Fig. 1.—Colonies of B. typhosus "Rawlings" on nutrient agar containing 1% xylose after 13 days' incubation.

Fig. 2.—Colonies of B. typhosus "Rawlings" on a control plate of the same nutrient agar without xylose.

Fig. 3.—Colonies of B. typhosus 156 on nutrient agar containing 1% arabinose after 13 days' incubation.

Fig. 4.—Colonies of B. typhosus 156 on nutrient agar containing 1% dulcite after 13 days' incubation.

PLATE I

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